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**PHYTOCHEMICAL CONSTITUENTS OF *GOMPHRENA SERRATA L***

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**ABSTRACT**

The aim of the study was to screen the phytoconstituents present in the flower extracts of *Gomphrena serrata L.* and their further analysis by GC-MS. The flowers of the plant were extracted using solvents hydroalcoholic mixture (50:50) and acetone. Preliminary phytochemical screening showed the presence of alkaloids, glycosides, tannins, flavonoids, steroids, amino acids and proteins. Column chromatography was carried out on the acetone extract of the plant. GC-MS analysis of chloroform fraction showed the presence of 30 bioactive compounds. The study forms a basis for the biological characterization and importance of the compounds identified.

**Keywords: *Gomphrena serrata*, GC-MS, bioactive compounds**

## 1. INTRODUCTION

Plants are the rich source of bioactive constituents with diverse pharmacological properties. The extraction and characterization of phytochemicals from plants have resulted in the discovery of novel drug entities with high therapeutic value (Reische DL, 1998). The plants of the genus *Gomphrena* belonging to family *Amaranthaceae* are employed in the treatment of various ailments like asthma, diarrhea, gastric disturbances (Vieira et al., 1994; Reische DL, 1998). The plant has been studied to possess antimalarial, carminative and diuretic properties (Gessler et al., 1994; Dhawan et al., 1977). Oleuropein has been isolated from the plant (Babu et al., 2012). The plant extracts have been studied to possess anticholinergic and antihistaminic properties (Vani et al., 2016). The present study has been undertaken to explore the phytoconstituents of the plant by GC-MS.

## 2. MATERIALS AND METHODS

### Collection of plant material

The plant material was collected from local grounds of Prasadampadu and Enikepadu coordinates 16°32'45"N 80°34'12"E of Vijayawada rural region, Krishna district, Andhra Pradesh, India. The plant specimen was identified and authenticated by Dr. P. Satya Narayana Raju, plant taxonomist,

Dept. of Botany & Microbiology, Acharya Nagarjuna University (ANU), Guntur (Dt), Andhra Pradesh, India. A voucher specimen 001/VIPW was deposited in the department of Pharmacognosy, Nirmala college of Pharmacy, Atmakur, Mangalagiri, A.P., India for future reference.

### Preparation of powder and extract

The flowers were dried under shade, powdered, coarsely using a mechanical grinder. Then the powder was extracted with 50:50 methanol, water and acetone alone using soxhlet apparatus. The extracts obtained were dried under vacuum, preserved in refrigerator for future use. The yield of extracts was found to be 25.0% w/w for hydroalcoholic mixture and 24.2% w/w for acetone solvent respectively (Evans et al., 2000).

### Preliminary Phytochemical screening

The plant extracts were tested for the presence of various phytochemicals by using standard methods (Evans et al., 2000).

### Isolation by column chromatography

Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with silica gel (Merck, Germany). Acetone extract of the plant (20 ml) was applied to the column by use of a pipette. It was eluted sequentially with

solvents in the increasing order of polarity. The chloroform fraction was collected and sent for GC-MS analysis (Taur et al., 2011; Laurence et al., 2001).

### GC-MS analysis

A Thermo GC-Trace Ultra Ver. 5.0, Thermo MS (Dual-Stage Quadrupole) DSQ II equipment (Thermo Scientific Co.) was used for carrying out the phytochemical investigation. Experimental conditions for GC-MS (Figure. 1) were DB 5-MS capillary standard non-polar column, dimension 30mts, ID 0.25mm, film thickness 0.25 $\mu$ m, carrier gas (mobile phase) helium was set at a flow rate of 1.0 ml/min, the oven temperature was 700C raised to 260  $^{\circ}$ C at 6  $^{\circ}$ C/min and injection volume was 1 $\mu$ l samples were dissolved in chloroform, run fully at a range of 50-650 m/z ( Manjamalai et al., 2011) .

### Characterization of compounds

Interpretation of mass spectrum of GC – MS was done using the database of National Institute of Standard and Technology (NIST4) and Wiley spectral library research program. The spectrum of the known components was compared with the spectrum of the known components stored in the inbuilt library. The name, molecular weight, and the structure of components were ascertained. The major components were given in table 1, with name, molecular formula, molecular weight, and percentage peak area, the mass spectra, fragmentation patterns and structures were depicted (Manjamalai et al., 2011) (Figure 2, Figure 3, Figure 4, Figure 5, Figure 6 and Figure 7) Other phytochemical components were given ( Table 2).

## 3. RESULTS & DISCUSSION

Table 1: Major chemical constituents of acetone extract from chloroform fraction of column by GC-MS analysis

S. No.	RT	Name of Phyto constituent	MF	MW	% Peak area
1.	6.71	14-Hydroxymorphinone - Semicarbazone	C18H22N4O3	342	0.47
2.	19.25	1-[[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-butadienyl)cyclopropane	C19H22O2S2	346	2.52
3.	21.63	Hexadecanoic acid, methyl ester	C17H34O2	270	5.92
4.	25.28	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296	8.80
5.	27.38	2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)-	C19H34O2	294	5.03
6.	31.19	Decahydro-8-[spiro-5'-bromo-3'-indolin-2'-one]-2-(p-tolyl)pyrrolo[3,4-a]pyrrolizine-1,3-dione	C22H18BrN3O3	451	38.56

Note: RT- Retention time; MF- Molecular formula; MW- Molecular weight

Table 2: Other chemical constituents of acetone extract from chloroform fraction of column by GC-MS analysis

S. no.	RT	Name of Phyto constituent	MF	MW	% Peak area
1.	4.08	1-Butanol, 2-amino-, (ñ)-	C4H11NO	89	0.25
2.	6.12	1,8-Dimethoxy-3-methylanthracene-9,10-dione	C17H14O4	282	0.26
3.	7.95	5',5',7'-Trimethyl-3'-(phenylmethyl)spiro[cyclopropane-1,9'-[8]oxa[3,7]diazabicyclo[4.2.1]nonane]	C18H26N2O	286	0.23
4.	9.07	1,2-Bis(2'-quinolylmethyl)ethylene	C20H14N2	282	0.41
5.	10.55	(+)-cis-5-[(tert-Butyldimethylsilyloxy)methyl]-7-methylidene-1,4-diphenyl-6,7-dihydro-5H-cyclopenta[d]pyridazine	C27H32N2OSi	428	0.38
6.	17.55	Tetradecanoic acid, methyl ester	C15H30O2	242	0.94
7.	18.96	methyl pentadecanoate / anteiso chain	C16H32O2	256	1.02
8.	20.18	3-Imino-2-phenylhydrazono-1,1,3-triphenyl-1-propanol	C27H23N3O	405	0.50
9.	20.82	(25R)-17á-Methyl-18-nor-17á-cholesta-5,13-diene-3á,26-diol	C27H44O2	400	0.90
10.	23.00	Hexadecanoic acid, 15-methyl-, methyl ester	C18H36O2	284	1.52
11.	23.46	2-Cyclopentene-1-undecanoic acid, methyl ester	C17H30O2	266	5.97
12.	24.91	(3R*,4S*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl)hexane	C19H23NO4	329	0.76
13.	26.74	bis(Timethylsilyl) 3,4-bis(methoxycarbonyl)hexanedioate	C16H30O8Si2	406	0.36
14.	28.88	Eicosanoic acid, methyl ester	C21H42O2	326	0.65
15.	29.74	1á-hydroxy-2-oxa-5á-lanost-8-en-3-one	C27H40O5	444	0.50
16.	30.45	2-(4',5'-Dimethyl-1',3'-diselenol-2'-ylidene)-1,3-dithio[4,5-d]-1,3-dithiol-2-one	C9H6OS4Se2	418	5.29
17.	32.57	2,6-Di-tert-butyl-4-[3,3-bis(4chlorophenyl)propadienyldiene]-2,5-cyclohexadien-1-one (Quinopropadiene)	C29H28Cl2O	462	1.53
18.	33.10	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	390	0.72
19.	34.41	Ethyl 5-hydroxy-8,9-methylenedioxy 12-methylbenzophenanthridine -5-carboxylate	C22H21NO5	379	1.73
20.	36.48	1-[(p-Yoluenesulfonyl)oxy]-3,8,12,16-Tetramethyl-3(E),7(E),11(E),15-heptadecatetraene	C28H42O3S	458	0.60
21.	37.85	3,4-bis[5'-(2''-Naphthyl)-2'-methylthiophen-3'-yl]-2,5-dihydro thiophene	C34H26S3	530	0.45
22.	38.60	4-toluene-d3-sulfonyl chloride	C7H4D3ClO2S	190	0.27
23.	39.03	9-Octadecenamide, (Z)-	C18H35NO	281	10.38
24.	39.93	Synaptogenin b	C30H46O4	470	3.10

Note: RT- Retention time; MF- Molecular formula; MW- Molecular weight

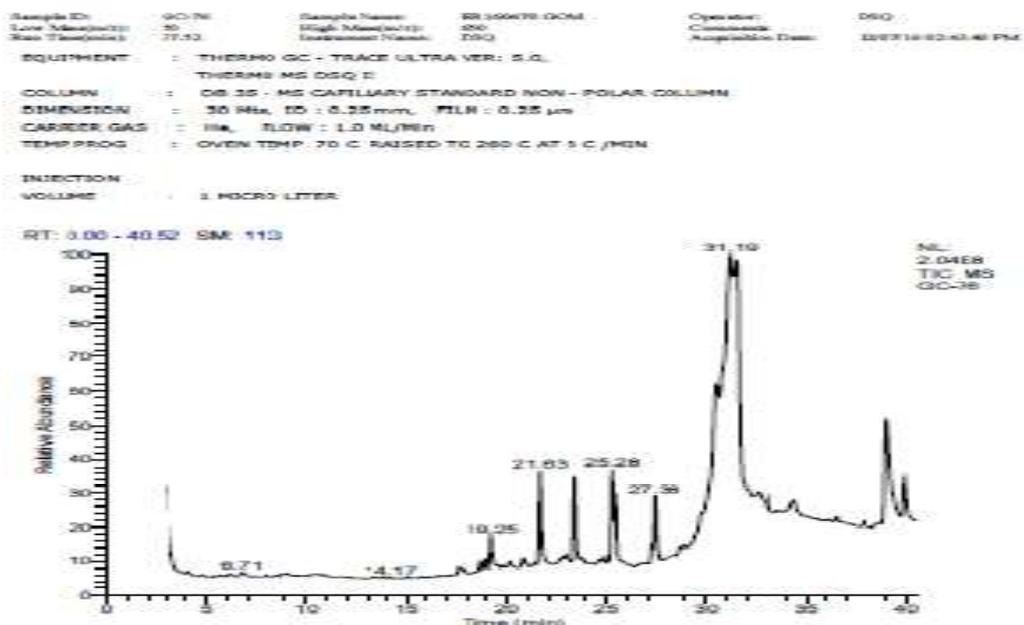
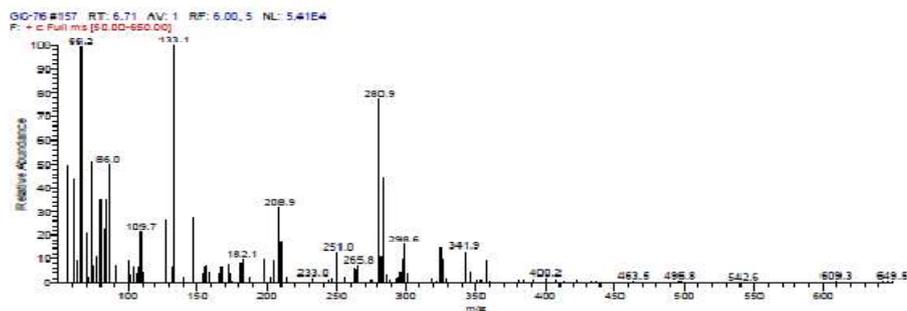


Figure 1-Gas chromatogram Mass spectrum of GSAE column fraction



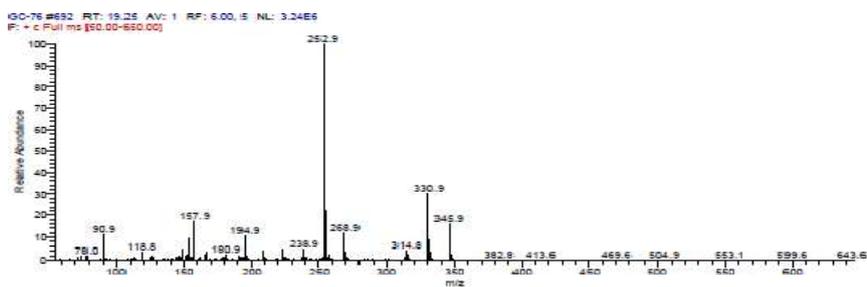
Library Search Results

SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
519	815	14-Hydroxymorphine - Semicarbazone	27.20	C18H22N4O3	342	0.47

Lib. Search Graphics Table

Compound Structure	Hit Spectrum
<p>14-Hydroxymorphine - Semicarbazone                      Formula C18H22N4O3, MW 342, CAS# NA, Entry# 457630</p>	<p>NI: 9.99E7                      CC: 76157 5.71 1                      RRF: 5.541E4 +c                      Full ms (50.00-650.00)</p> <p>NL: 9.99E2                      SI: 519, RSI: 815,                      Wiley8, Entry# 457630,                      CAS# NA,                      14-Hydroxymorphine - Semicarbazone</p>

Figure 2 - Mass spectrum at RT 6.71



Library Search Results						
SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
958	958	1-[[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-butadienyl)cyclopropane	76.25	C19H22O2S2	346	2.52

Lib. Search Graphics Table

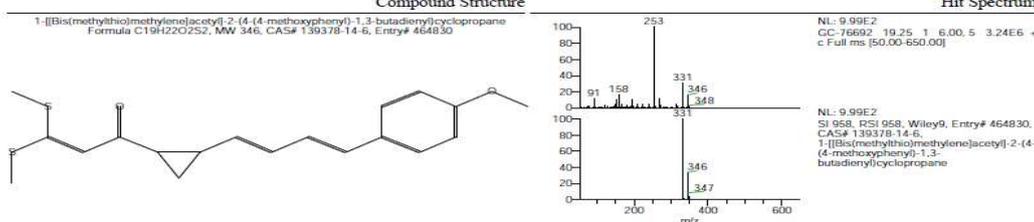
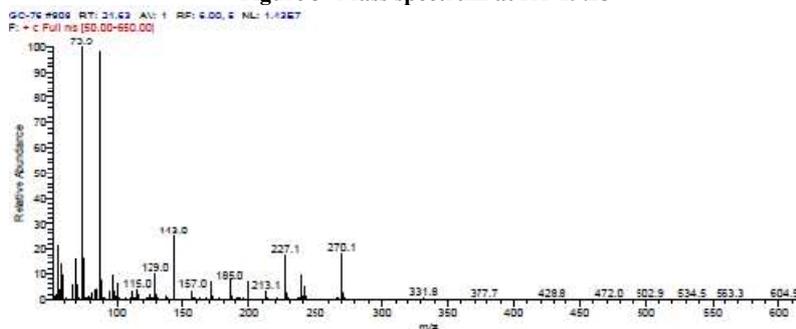


Figure 3- Mass spectrum at RT 19.25



Library Search Results						
SI	RSI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
901	916	Hexadecanoic acid, methyl ester (CAS)	59.24	C17H34O2	270	5.92

Lib. Search Graphics Table

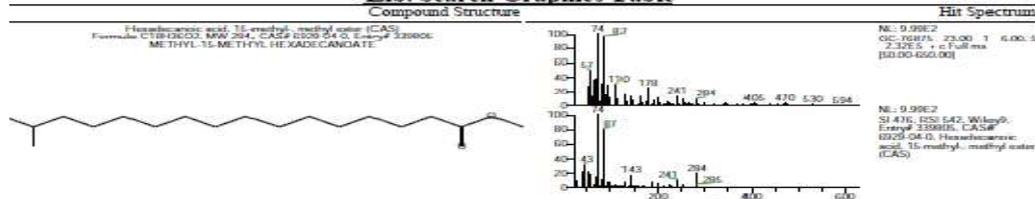


Figure 4 - Mass spectrum at RT 21.63

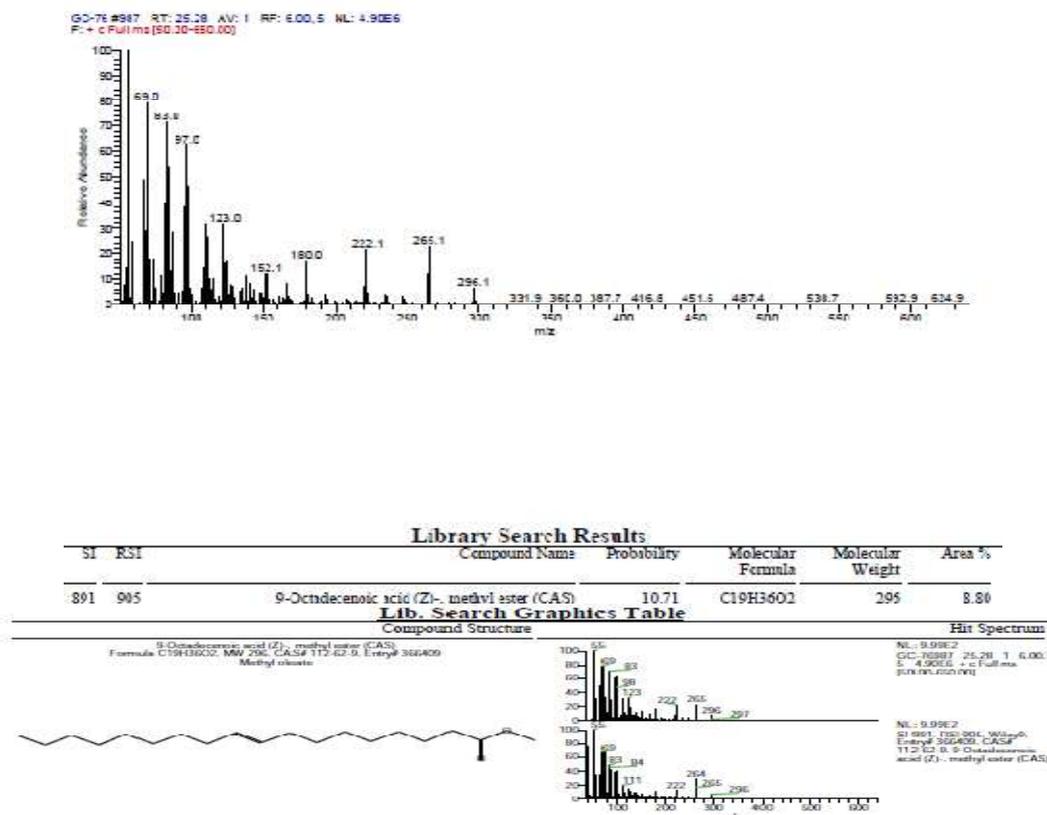


Figure 5 - Mass spectrum at RT 25.28

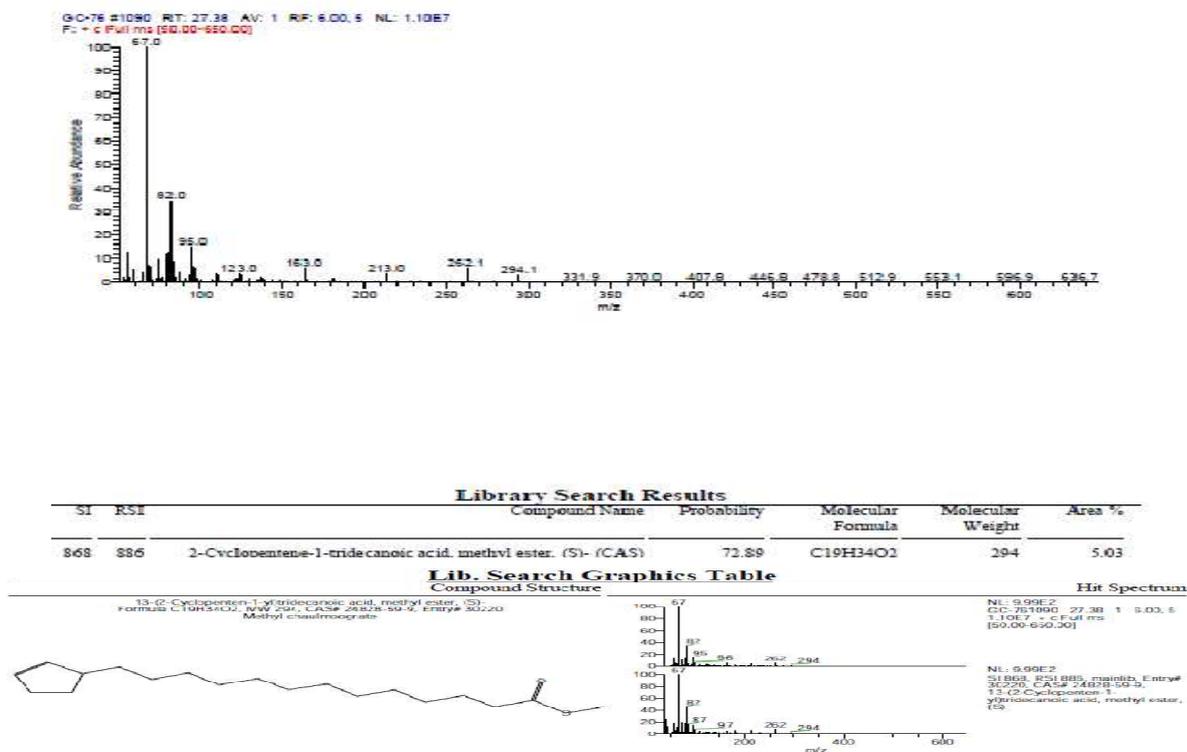
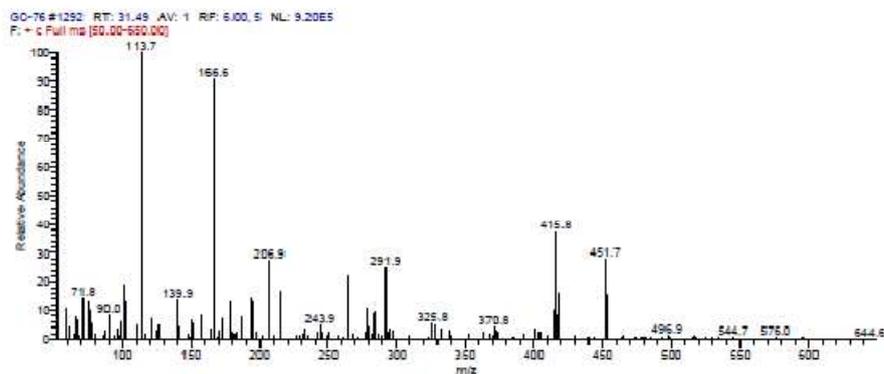


Figure 6 - Mass spectrum at RT 27.38



## Library Search Results

SI	ESI	Compound Name	Probability	Molecular Formula	Molecular Weight	Area %
656	744	Decahydro-8-[spiro-5'-bromo-3'-indolin-2'-one]-2-(p-tolyl)pyrrolo[3,4-a]pyrrolizine-1,3-dione	66.30	C <sub>22</sub> H <sub>18</sub> BrN <sub>3</sub> O <sub>3</sub>	451	38.56

## Lib. Search Graphics Table

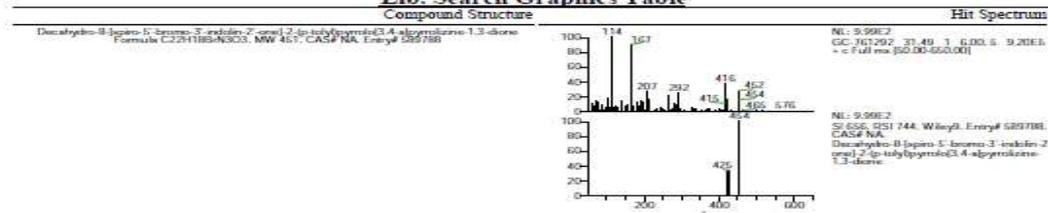


Figure 7- Mass spectrum at RT 31.19

Table 3: Activity of phytoconstituents identified in the acetone extract from chloroform fraction of column by GC-MS analysis

S. No.	Name of Phyto constituent	Nature of Phyto constituent	Activity
1.	14-Hydroxymorphinone – Semicarbazone	Glucuronide metabolite	Not reported
2.	1-[[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-butadienyl)cyclopropane	Aromatic hydrocarbon	Antimicrobial
3.	Hexadecanoic acid, methyl ester	Palmitic acid	Antioxidant antiandrogenic
4.	9-Octadecenoic acid (Z)-methyl ester	Fatty acid ester	Antioxidant Antimicrobial Anticarcinogenic
5.	2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)-	Fatty acid	Not reported
6.	Decahydro-8-[spiro-5'-bromo-3'-indolin-2'-one]-2-(p-tolyl)pyrrolo[3,4-a]pyrrolizine-1,3-dione	Alkaloid	Not reported
7.	1-Butanol, 2-amino-, (n)-	Aliphatic alcohol	Anaesthetic Haemodynamic Antiarrhythmic Hypotensive Antimalarial
8.	1,8-Dimethoxy-3-methylanthracene-9,10-dione	Aromatic ketone	Antibacterial
9.	5',5',7'-Trimethyl-3'-(phenylmethyl)spiro[cyclopropane-1,9'-[8]oxa[3,7]diazabicyclo[4.2.1]nonane]	Aliphatic compounds	Not reported
10.	1,2-Bis(2'-quinolylmethyl)ethylene	Alkaloid	Not reported
11.	(+)-cis-5-(tert-	Alkaloid	Not reported

	Butyldimethylsilyloxy)methyl]-7-methylidene-1,4-diphenyl-6,7-dihydro-5H-cyclopenta[d]pyridazine		
12.	Tetradecanoic acid, methyl ester	Fatty acid	Antioxidant, Anticancer Nematicide Hypercholesterolemic Lubricant
13.	methyl pentadecanoate / anteiso chain	Fatty acid	Not reported
14.	3-Imino-2-phenylhydrazono-1,1,3-triphenyl-1-propanol	Triphenyl alcohols	Inhibitors of <i>Plasmodium falciparum</i> Inhibitors of human mitotic kinesin
15.	(25R)-17 $\alpha$ -Methyl-18-nor-17 $\alpha$ -cholesta-5,13-diene-3 $\alpha$ ,26-diol	Oxy sterols	Regulators of cholesterol homeostasis
16.	Hexadecanoic acid, 15-methyl-, methyl ester	Fatty acid	Antioxidant Antiandrogenic Antihistaminic Hepatoprotective
17.	2-Cyclopentene-1-undecanoic acid, methyl ester	Fatty acid	Not reported
18.	(3R*,4S*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl)hexane	Aromatic compound	Not reported
19.	bis(Timethylsilyl) 3,4-bis(methoxycarbonyl)hexanedioate	Aliphatic compounds	Not reported
20.	Eicosanoic acid, methyl ester	Saturated fatty acid	Anticancer, Causes increased levels of serum low-density lipoproteins in human body
21.	1 $\alpha$ -hydroxy-2-oxa-5 $\alpha$ -lanost-8-en-3-one	Steroid	Not reported
22.	2-(4',5'-Dimethyl-1',3'-diselenol-2'-ylidene)-1,3-dithio[4,5-d]-1,3-dithiol-2-one	Aromatic compound	Not reported
23.	2,6-Di-tert-butyl-4-[3,3-bis(4chlorophenyl)propadienylidene]-2,5-cyclohexadien-1-one (Quinopropadiene)	Aromatic hydrocarbon	Not reported
24.	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Aromatic acidic compound	Not reported
25.	Ethyl 5-hydroxy-8,9-methylenedioxy-12-methylbenzophenanthridine -5-carboxylate	Phenanthrene	Not reported
26.	1-[(p-Yoluenesulfonyloxy]-3,8,12,16-Tetramethyl-3(E),7(E),11(E),15-heptadecatetraene	Aromatic hydrocarbon with sulphur and oxygen	Not reported
27.	3,4-bis[5'-(2''-Naphthyl)-2'-methylthiophen-3'-yl]-2,5-dihydro thiophene	Aromatic amine	Antioxidant
28.	4-toluene-d3-sulfonyl chloride	Aliphatic hydrocarbon Compound	Not reported
29.	9-Octadecenamide, (Z)-	Aliphatic amide compound	Antimicrobial
30.	Synaptogenin b	Artefactual aglycone	Antimicrobial

Note: Source - Dr. Duke's Phytochemical and Ethnobotanical database.

The identified compounds from the GC-MS were 14-Hydroxymorphinone – Semicarbazone (0.47%), 1-[[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-tadienyl)cyclopropane (2.52%), Hexadecanoic acid, methyl ester (5.92%), 9-Octadecenoic acid (Z)-, methyl ester (8.80%), 2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)- (5.03%) , Decahydro-8-[spiro-5'-bromo-3'-indolin-2'-one]-2-(p-tolyl)pyrrolo[3,4-a]pyrrolizine-1,3-dione (38.56%), 1-Butanol, 2-amino-( $\bar{n}$ )- (0.25%), 1,8-Dimethoxy-3-methylantracene-9,10-dione (0.26%), 5',5',7'-Trimethyl-3'-(phenylmethyl)spiro[cyclopropane-1,9'-[8]oxa [3,7] diazabicyclo [4.2.1]nonane](0.23%), 1,2-Bis(2'-quinolylmethyl)ethylene(0.41%) (Table 1 and Table 2).

(+)-cis-5-[(tert-Butyldimethylsilyloxy)methyl]-7-methylidene-1,4-diphenyl-6,7-dihydro-5H-cyclopenta[d]pyridazine (0.38%), tetradecanoic acid, methyl ester (0.94%), methyl pentadecanoate / anteiso chain (1.02%), 3-Imino-2-phenylhydrazono-1,1,3-triphenyl-1-propanol (0.50%), (25R)-17 $\alpha$ -Methyl-18-nor-17 $\alpha$ -cholesta-5,13-diene-3 $\alpha$ ,26-diol (0.90%), hexadecanoic acid, 15-methyl-, methyl ester (1.52%), 2-Cyclopentene-1-undecanoic acid, methyl ester (5.97%), (3R\*,4S\*)-3-(2-Nitro-4-methoxyphenyl)-4-(4-hydroxyphenyl)hexane (0.76%), bis (Trimethylsilyl) 3,4-bis(methoxycarbonyl)hexanedioate (0.36%), eicosanoic acid methyl ester, (0.65%), 1 $\alpha$ -hydroxy-2-oxa-5 $\alpha$ -lanost-8-en-3-one (0.50%), 2-(4',5'-Dimethyl-1',3'-diselenol-2'-ylidene)-1,3-dithio[4,5-d]-1,3-dithiol-2-one (5.29%) (Table 1 and Table 2).

2,6-Di-tert-butyl-4-[3,3-bis(4-chlorophenyl)propadienylydene]-2,5-cyclohexadien-1-one (Quinopropadiene) (1.53%), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (0.72%), Ethyl 5-hydroxy-8,9-methylenedioxy-12-methylbenzophenanthridine -5-carboxylate (1.73%), 1-[(p-Toluenesulfonyl)oxy]-3,8,12,16-Tetramethyl-3(E),7(E),11(E),15-heptadecatetraene (0.60%), 3,4-bis[5'-(2"-

Naphthyl)-2'-methylthiophen-3'-yl]-2,5-dihydrothiophene (0.45%), 4-toluene-d3-sulfonyl chloride (0.27%), 9-Octadecenamide, (Z) (10.38%), and synaptogenin B (3.10%) (Table 1 and Table 2).

There is a growing awareness in correlating the phytochemical components and their biological activities (Ferne et al., 2004; Sumner et al., 2003; Robertson 2005). In the present study, GC-MS analysis showed the presence of 30 compounds. The pharmacological applications of these compounds were given in table 3 (Dr. Duke's Phytochemical and Ethnobotanical database). In respect of percentage peak area decahydro-8-[spiro-5'-bromo-3'-indolin-2'-one]-2-(p-tolyl) pyrrolo [3, 4-a] pyrrolizine-1, 3-dione (38.56%), an alkaloid was predominant. It was not reported for any biological activity to date. The next dominant phytochemicals were saturated fatty acids, needed for energy, hormone production, cellular membranes and organ padding. They also serve the important signaling and stabilization processes in the body (Mary Eng). 9-Octadecenamide, (Z) (10.38%) was reported for antimicrobial activity, and 9-Octadecenoic acid (Z)-, methyl ester (8.80%) (Table 1, Table 2 & Table 3) was reported to exhibit antioxidant,

antimicrobial and anticarcinogenic activities. Octadecanoic acid is also called as the stearic acid was reported to have effects equivalent to oleic acid in lowering plasma cholesterol levels (Andrea et al., 1998).

The other compounds with considerable peak area include, 2-Cyclopentene-1-undecanoic acid, methyl ester (5.97%) was reported for no biological activity, hexadecanoic acid, methyl ester (5.92%) was reported to possess antioxidant and antiandrogenic activities. Hexadecanoic acid is also called as Palmitic acid strongly boosts among all fatty acids metastatic potential of CD36<sup>+</sup> metastasis-initiating cells, (Gloria et al., 2016; Sanchez 2016) showed alterations in central nervous system control of insulin secretion (Fabiana 2013), and suppression of the body's natural appetite-suppressing signals from leptin and insulin (the key hormones involved in weight regulation) (Benoit et al., 2009).

Tetradecanoic acid, methyl ester (0.94%), was reported to possess antioxidant, anticancer, nematicide hypercholesterolemic and lubricant effects. Tetradecanoic acid is also known as myristic acid, a very important fatty acid, which the body uses to stabilize many different proteins, including proteins used in the immune system and to fight tumors. This function is called myristoylation; the loss of myristic acid from

the diet can have unfortunate consequences, including cancer and immune system dysfunction (Mary Enig 2004). 2-(4', 5'-Dimethyl-1',3'-diselenol-2'-ylidene)-1,3-dithio[4,5-d]-1,3-dithiol-2-one (5.29%) was not reported to possess any biological activities, 2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)- (5.03%) was not reported for biological activities, 1-[[Bis(methylation)methylene]acetyl]-2-(4-(4-methoxyphenyl)-1,3-tadienyl)cyclopropane (2.52%) was reported to possess antimicrobial activity, and an artefactual aglycone synaptogenin B (3.10%) was reported to possess antimicrobial activity (Table 1, Table 2 & Table 3) Thus phytochemical analysis formed a platform for future studies on the plant extracts of *Gomphrena serrata* L.

#### 4. CONCLUSION

GC-MS analysis of the chloroform fraction of acetone extract of *Gomphrena serrata* reveals the presence of medicinally valued bioactive components. Further isolation and study of activities of individual components would brighten the pharmacological profile of the plant.

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